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Title

Experimental Emphysema : The Effects of Prolonged Dust and Nitrogen Dioxide Exposure on the Physiologic and Morphometric Parameters of the Hamster Lung

Progress Report

The project entitled, "Experimental Emphysema : The Effect of Prolonged Dust and Nitrogen Dioxide Exposure on the Physiologic and Morphometric Parameters of the Hamster Lung" has been operational from July 1, 1972. This report covers the period through December 31, 1972. During this period the exposures have been initiated and are in progress. This has entailed development of dust disseminating procedures and methods of measuring dust concentrations simultaneously with the evolution of Nitrogen Dioxide. Three groups of 36 male Syrian hamsters weighing approximately 80 Gm. each and housed separately in gerbil cages have been exposed as follows : Group 1 Exposed to respirable fly ash gathered from the electrostatic precipitators of the Cleveland Electric Illuminating Co. The total dust concentration in chamber A for the six month period is 3.21 mg/cu. m. (S. D. ± 0.96; S. E.±0.39). The respirable dust collected by elutriator during the same period has been 0.84 mg/cu. m. (S. D. ± 0.35; S. E.±0.14). In chamber B the six month mean total dust concentration was 4.35 mg/ cu. m. (S. D. ± 1.24; S. E.± 0.50); the mean respirable dust concentration in this chamber was 0.83 mg/cu. m. (S. D. ± 0.13; S. E.± 0.05). Dust concentrations vary according to the site at which the collection is made. This has been standardized as much as possible by placing the collecting millipore filters and elutriators at sites of comparable dust concentration and maintaining these collection sites continuously. Samples are collected five days per week for periods of 4-6 hours; exposures are performed seven days per week.

The mean Nitrogen Dioxide concentrations during the first six months is 207 ppm (S. D. ± 0.38; S. E. ± 0.15). Nitrogen Dioxide concentrations are recorded continuously by mast meter on a Honeywell-Brown recorder. Nitrogen Dioxide exposures are performed seven days weekly for 20-22 hours daily. The mast meter readings are calibrated twice daily Monday through Friday and once only on Saturday and Sunday. Calibration is by the Saltzmann method of Nitrogen Dioxide analysis.

The mean weight of the animals exposed to dust and Nitrogen Dioxide, Group 2 has increased from 74 ± 7 Gm. to 124.5 ± 14 Gm. in a six month period. In the same period hamsters exposed to dust only have gone from their original mean weight of 77.1 ± 6.83 Gm. to 124.76 ± 12.55 Gm. The values for the control (unexposed) group, Group 3, are as follows : Mean initial weight 80 ± 9 Gm; at the end of six months 124.2 ± 12.54. Thus the exposures do not seem to have interfered with the usual growth and weight increase as compared with the controls.

A number of animals have died of a variety of causes. Several have developed what appears to be abscesses in sinuses, orbit and maxilla which progress to intracranial pyogenic infections either meningitis or subdural abscesses. On several occasions cultures taken at autopsy have demonstrated a variety of organisms including E. Coli. Pseudomonas, anerobic streptococci and monilia. Other causes of death include nephrosis and anasarca, or trauma. In other cases cannabalization prevented autopsy study. The mortality during the six month period of the experiment has been none in the dust group, seven in the dust Nitrogen Dioxide exposures and six in the control.

Preliminary experiments have been performed in order to develop and perfect the methods of physiologic study. In a series of normal control animals the following physiologic data has been collected.

Ambient Air : n = 9, Static compliance = .526 ml/cm H_2O

Dynamic compliance at 20 cpm .295 cc/cm H_2O
50 cpm .266 cc/cm H_2O
80 cpm .250 cc/cm H_2O

Studies during natural deflation :

Expiratory flow rate at 2 cc lung volume (cc) 14.1 ± 2.52, S. E. 0.89

1 cc lung volume (cc) 7.89 + 1.59, S. E. 0.56

Total pulmonary resistance at 2 cc 1.88 cm H_2O/ml/sec ± .62 S. E..23

at 1 cc 1.90 cm H_2O/ml/sec ± .48 S. E. .18

Studies during forced deflation at -50 cm. water:

Max. Expiratory flow rate at 2 cc lung volume 34.78 ± 6.16 SE (2.05)

at 1 cc lung volume 15.32 ± 5.34 SE (1.78)

Upstream resistance at 2 cc lung volume 1.24 ± .40 SE (0.13)

at 1 cc lung volume 1.52 ± 0.72 SE (.24)

These studies indicate that the methods for measurement of postmortem pulmonary function are reliable and reproducible for our studies.

Measurement of mean linear intercept, and internal surface area of the lung has been performed in a series of control animals. These measurements performed by human eye have the following values : Mean Linear Intercept (L_m) .0718 ± .0030 mm and internal surface area (ISA) 2,330 ± 390 cm^2. In addition efforts have been initiated to develop automated techniques for measurement of L_m and ISA. The quantimet image analyzing computer has been utilized for these measurements. In a series of comparisons the L_m and ISA as measured by quantimet at a magnification of 80 times were L_m .0685/mm and ISA 2760 cm^2 while at a magnification of 200 times measurement values were L_m .0601/mm^2 and ISA 3150 cm^2, as compared to values measured by human eye in the same animal lung of L_m.0678/mm and ISA 2,650 cm^2.

These data suggest that the morphometric evaluation of the hamster lungs may be adaptable to semi-automated techniques using the Quantimet. This could be a considerable boon in terms of time saving and avoidance of terribly tedious and exacting measurements.

Preliminary measurements of ISA have been performed in this laboratory by the manual method described by Thurlbeck and compared to values obtained by an automated method using the Quantimet 720. The procedure used for comparing these two methods is as follows : A histologic slide of lung tissue (6 micron) stained with hematoxylin-eosin was overlayed with a grid of 2 mm squares. The grid allows for systematic scanning of the section without inadvertently duplicating fields for measurement. By using machine modules which house a shading corrector and a module capable of projecting a precalibrated variable measuring frame, alveolar wall intercepts were machine counted.Manual intercept counts were performed on the projected image on the television monitor from two of the five projected precalibrated lines. Fifty random fields were counted avoidingareas which con-contained large vessels and bronchi. An analysis of variance was applied to test for significant differences between machine and manual counts on the television screen. When 50 automated counts were compared to the manuall counts the p value is <.60, no significant difference. When 50 frame manual counts on the TV monitor are compared to 125 machine counts which included the same field, the p value was <.90 again indicating no significant difference but a closer association of values. Since the time required to increase the number of quantimet measurements is insignificant, the protocol will stipulate 125 variable frame counts measured.

Staining procedures and Quantimet 720 measurement methodologies have also been devised for the counting of goblet cells and for determining the ratio of total (area) gland mass to total area of the tracheal or bronchial section. Histologic sections (6 micron) stained with Alcian blue and Schiff reagent (PAS) will be used to count goblet cells, accumulate the area positively stained by the Alcian blue - PAS. These data will be related to the total area measurement of the section. Tracheal and bronchial sections stained with Alcian blue and with PAS will be used to measure the ratio of total area of gland mass to total area of the section. This will be accomplished utilizing the light pen module of the Quantimet 720, which can measure and accumulate data on isolated features in the optical field.